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APPLICANT: Stanley T. Crooke  
SERIAL NO: 10/078,949

DOCKET NO: ISIS-5027

**AMENDMENTS TO THE CLAIMS:** This listing of claims replaces all prior versions and listings of claims in the instant patent application.

**Listing of claims:**

1-164. (Canceled)

165. (Currently amended) A method of activating a double-stranded RNA nuclease, comprising comprising:

(i) contacting the nuclease with a double-stranded RNA comprising a first oligonucleotide and a second oligonucleotide, wherein:

at least one of said first and said second oligonucleotides comprise comprises at least four consecutive 2'-hydroxyl ribonucleosides and at least one modified nucleoside chemical modification;

said first and said second oligonucleotides are hybridized to each other; and

said first and said second oligonucleotides are not covalently linked; and

(ii) detecting activation of said double-stranded RNA nuclease.

166. (Canceled)

167. (Currently amended) The method of claim 165, wherein the modified nucleoside or nucleosides chemical modifications increase resistance of said oligonucleotide to single-stranded nucleases and/or increase the affinity of said oligonucleotide to the other oligonucleotide.

168. (Previously presented) The method of claim 167, wherein at least one modification is 2'-methoxy.

169. (Previously presented) The method of claim 167, wherein at least one modification is 2'-fluoro.

170. (Previously presented) The method of claim 167, wherein at least one modification is 2'-O-(methoxyethyl).

171. (Previously presented) The method of claim 167, wherein at least one

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modification is a phosphorothioate internucleoside linkage.

172. (Previously presented) The method of claim 165, wherein said first oligonucleotide and said second oligonucleotide each have at least four consecutive 2'-hydroxyl ribonucleosides.

173. (Previously presented) The method of claim 172, wherein the 2'-hydroxyl residues of said first and said second oligonucleotides have phosphodiester linkages.

174. (Previously presented) The method of claim 172, wherein the 2'-hydroxyl residues of said first and said second oligonucleotides have phosphorothioate linkages.

175. (Previously presented) The method of claim 172, wherein the 2'-hydroxyl residues of said first oligonucleotide have phosphodiester linkages and the 2'-hydroxyl residues of said second oligonucleotide have phosphorothioate linkages.

176. (Previously presented) The method of claim 172 or claim 175, wherein said first and said second oligonucleotides further comprise flanking residues 5' and 3' of the 2'-hydroxyl ribonucleosides, wherein said flanking residues have phosphorothioate linkages.

177. (Previously presented) The method of claim 176, wherein said flanking residues of at least one of said first and said second oligonucleotides further comprises 2'-methoxynucleosides.

178. (Previously presented) The method of claim 176, wherein said flanking residues of each of said first and said second oligonucleotides further comprise 2'-methoxynucleosides.

179. (Previously presented) The method of claim 165, wherein at least one of said first and said second oligonucleotides comprises at least eight consecutive 2'-hydroxyl ribonucleosides.

180. (Previously presented) The method of claim 179, wherein said first oligonucleotide and said second oligonucleotide each comprise at least eight consecutive 2'-hydroxyl ribonucleotides.

181. (Previously presented) The method of claim 165, wherein each of said first and

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said second oligonucleotides are about 17 to about 20 nucleoside subunits in length.

182. (Previously presented) The method of claim 181, wherein each of said first and said second oligonucleotides are 17 subunits in length.

183. (Previously presented) The method of claim 181, wherein each of said first and said second oligonucleotides are 20 subunits in length.

184-201. (Canceled)

202. (New) A method of activating a double-stranded RNA nuclease comprising contacting the nuclease with a double-stranded RNA comprising a first oligonucleotide and a second oligonucleotide, wherein:

said first and said second oligonucleotides are independently 15 to 25 nucleoside subunits in length;

said first and said second oligonucleotides are hybridized to each other;

said first and said second oligonucleotides are not covalently linked; and

at least one of said first and said second oligonucleotides comprises at least four consecutive 2'-hydroxyl ribonucleosides and at least one chemical modification.

203. (New) The method of claim 202 wherein at least one chemical modification increases resistance to single-stranded nucleases.

204. (New) The method of claim 202 wherein at least one chemical modification increases affinity of said first oligonucleotide to said second oligonucleotide.

205. (New) The method of claim 202 wherein at least one at least one chemical modification is a modified internucleoside linkage, a modified sugar moiety or a modified nucleobase.

206. (New) The method of claim 202 wherein at least one chemical modification is a phosphorothioate internucleoside linkage.

207. (New) The method of claim 202 wherein at least one chemical modification is a 2'-substituted sugar modification.

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208. (New) The method of claim 202 wherein at least one chemical modification is a 2'-alkoxy sugar modification.

209. (New) The method of claim 202 wherein at least one chemical modification is a 2'-methoxy sugar modification.

210. (New) The method of claim 202 wherein at least one chemical modification is a 2'-fluoro sugar modification.

211. (New) The method of claim 202 wherein at least one chemical modification is a 2'-O-methoxyethyl sugar modification.

212. (New) The method of claim 202 wherein each of said first and said second oligonucleotides comprises at least four consecutive 2'-hydroxyl ribonucleosides.

213. (New) The method of claim 202 wherein each of said first and said second oligonucleotides comprises at least one chemical modification.

214. (New) The method of claim 202 wherein each of said first and said second oligonucleotides comprises at least four consecutive 2'-hydroxyl ribonucleosides and at least one chemical modification.

215. (New) The method of claim 202 wherein said first oligonucleotide and said second oligonucleotide comprise at least 17 contiguous nucleotides which are 100% complementary to each other.

216. (New) The method of claim 202 wherein said first oligonucleotide is 100% complementary to said second oligonucleotide.

217. (New) The method of claim 202 wherein said first oligonucleotide and said second oligonucleotide are independently 17 to 20 nucleoside subunits in length.

218. (New) The method of claim 202 further comprising detecting activation of said double-stranded RNA nuclease.

219. (New) A method of activating a double-stranded RNA nuclease comprising contacting the nuclease with a double-stranded RNA comprising a first oligonucleotide and a

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second oligonucleotide, wherein:

said first and said second oligonucleotides are independently 15 to 25 nucleoside subunits in length;

said first and said second oligonucleotides are hybridized to each other;

said first and said second oligonucleotides are not covalently linked; and

at least one of said first and said second oligonucleotides comprises a plurality of nucleoside subunits with 2'-hydroxyl pentofuranosyl sugar moieties and at least one chemical modification.

220. (New) The method of claim 219 wherein at least one chemical modification increases resistance to single-stranded nucleases.

221. (New) The method of claim 219 wherein at least one chemical modification increases affinity of said first oligonucleotide to said second oligonucleotide.

222. (New) The method of claim 219 wherein at least one chemical modification is a modified internucleoside linkage, a modified sugar moiety or a modified nucleobase.

223. (New) The method of claim 219 wherein at least one chemical modification is a phosphorothioate internucleoside linkage.

224. (New) The method of claim 219 wherein at least one chemical modification is a 2'-substituted sugar modification.

225. (New) The method of claim 219 wherein at least one chemical modification is a 2'-alkoxy sugar modification.

226. (New) The method of claim 219 wherein at least one chemical modification is a 2'-methoxy sugar modification.

227. (New) The method of claim 219 wherein at least one chemical modification is a 2'-fluoro sugar modification.

228. (New) The method of claim 219 wherein at least one chemical modification is a 2'-O-methoxyethyl sugar modification.

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229. (New) The method of claim 219 wherein each of said first and said second oligonucleotides comprises a plurality of nucleoside subunits with 2'-hydroxyl pentofuranosyl sugar moieties.

230. (New) The method of claim 219 wherein each of said first and said second oligonucleotides comprises at least one chemical modification.

231. (New) The method of claim 219 wherein each of said first and said second oligonucleotides comprises a plurality of nucleoside subunits with 2'-hydroxyl pentofuranosyl sugar moieties and at least one chemical modification.

232. (New) The method of claim 219 wherein said first oligonucleotide and said second oligonucleotide comprise at least 17 contiguous nucleotides which are 100% complementary to each other.

233. (New) The method of claim 219 wherein said first oligonucleotide is 100% complementary to said second oligonucleotide.

234. (New) The method of claim 219 wherein said first oligonucleotide and said second oligonucleotide are independently 17 to 20 nucleoside subunits in length.

235. (New) The method of claim 219 further comprising detecting activation of said double-stranded RNA nuclease.

236. (New) A method of activating a double-stranded RNA nuclease comprising contacting the nuclease with a double-stranded RNA comprising a first oligonucleotide and a second oligonucleotide, wherein:

    said first and said second oligonucleotides are hybridized to each other;  
    said first and said second oligonucleotides are not covalently linked; and  
    each of said first and said second oligonucleotides comprises at least four consecutive 2'-hydroxyl ribonucleosides and at least one chemical modification.

237. (New) The method of claim 236 whercin at least one chemical modification increases resistance to single-stranded nucleases.

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238. (New) The method of claim 236 wherein at least one chemical modification increases affinity of said first oligonucleotide to said second oligonucleotide.

239. (New) The method of claim 236 wherein at least one chemical modification is a modified internucleoside linkage, a modified sugar moiety or a modified nucleobase.

240. (New) The method of claim 236 wherein at least one chemical modification is a phosphorothioate internucleoside linkage.

241. (New) The method of claim 236 wherein at least one chemical modification is a 2'-substituted sugar modification.

242. (New) The method of claim 236 wherein at least one chemical modification is a 2'-alkoxy sugar modification.

243. (New) The method of claim 236 wherein at least one chemical modification is a 2'-methoxy sugar modification.

244. (New) The method of claim 236 wherein at least one chemical modification is a 2'-fluoro sugar modification.

245. (New) The method of claim 236 wherein at least one chemical modification is a 2'-O-methoxyethyl sugar modification.

246. (New) The method of claim 236 further comprising detecting activation of said double-stranded RNA nuclease.

247. (New) A method of activating a double-stranded RNA nuclease comprising contacting the nuclease with a double-stranded RNA comprising a first oligonucleotide and a second oligonucleotide, wherein:

    said first and said second oligonucleotides are hybridized to each other;

    said first and said second oligonucleotides are not covalently linked;

    said first and said second oligonucleotides are 100% complementary to each other; and

    at least one of said first and said second oligonucleotides comprises at least four

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consecutive 2'-hydroxyl ribonucleosides and at least one chemical modification.

248. (New) The method of claim 247 wherein at least one chemical modification increases resistance to single-stranded nucleases.

249. (New) The method of claim 247 wherein at least one chemical modification increases affinity of said first oligonucleotide to said second oligonucleotide.

250. (New) The method of claim 247 wherein at least one chemical modification is a modified internucleoside linkage, a modified sugar moiety or a modified nucleobase.

251. (New) The method of claim 247 wherein at least one chemical modification is a phosphorothioate internucleoside linkage.

252. (New) The method of claim 247 wherein at least one chemical modification is a 2'-substituted sugar modification.

253. (New) The method of claim 247 wherein at least one chemical modification is a 2'-alkoxy sugar modification.

254. (New) The method of claim 247 wherein at least one chemical modification is a 2'-methoxy sugar modification.

255. (New) The method of claim 247 wherein at least one chemical modification is a 2'-fluoro sugar modification.

256. (New) The method of claim 247 wherein at least one chemical modification is a 2'-O-methoxyethyl sugar modification.

257. (New) The method of claim 247 further comprising detecting activation of said double-stranded RNA nuclease.